CLEAN VERSION WITH CHANGES INCORPORATED

In the Specification

The following text replaces the text previously found in page 58, line 12 to page 69, line 4:

"The present invention provides for a method for producing a protein of choice comprising a lysosomal enzyme which is enzymatically active, comprising: recovering the lysosomal enzyme from (i) a transgenic plant cell or (ii) a cell, tissue or organ of a transgenic plant, which transgenic plant cell or plant is transformed or transfected with a recombinant expression construct comprising a nucleotide sequence encoding the lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence so that the lysosomal enzyme is expressed by the transgenic plant cell or plant. The promoter can be an inducible promoter. The inducible promoter can be induced by mechanical gene activation. The method can be carried out with the transgenic plant and additionally comprises a step of inducing the inducible promoter before or after the transgenic plant is harvested, which inducing step is carried out before recovering the lysosomal enzyme from the cell, tissue or organ of the transgenic plant. The lysosomal enzyme can be a modified lysosomal enzyme which is enzymatically active and comprises: (a) an enzymatically-active fragment of a human or animal lysosomal enzyme; (b) the human or animal lysosomal enzyme or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human or animal lysosomal enzyme or (a); or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can comprise a signal peptide or detectable marker peptide at the amino or carboxyl terminal of the modified lysosomal enzyme. The modified lysosomal enzyme can be recovered from (i) the transgenic plant cell or (ii) the cell, tissue or organ of the transgenic plant by reacting with an antibody that binds the detectable marker peptide. The antibody can be a monoclonal antibody. The modified lysosomal enzyme

mannosidase or sialidase; (b) the *a*-N-acetylgalactosaminidase, acid lipase, *a*-galactosidase, glucocerebrosidase, *a*-L-iduronidase, iduronate sulfatase, *a*-mannosidase, sialidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the *a*-N-acetylgalactosaminidase, acid lipase, *a*-galactosidase, glucocerebrosidase, *a*-L-iduronidase, iduronate sulfatase, *a* -mannosidase, sialidase or (a); or (c) the *a*-N-acetylgalactosaminidase, acid lipase, *a*-galactosidase, glucocerebrosidase, *a*-L-iduronidase, iduronate sulfatase, *a*-mannosidase, sialidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can comprise: (a) an enzymatically-active fragment of a human glucocerebrosidase or human α-L-iduronidase enzyme; (b) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human glucocerebrosidase, human α-L-iduronidase or (a); or (c) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can be a fusion protein comprising: (I) (a) the enzymatically-active fragment of the human or animal lysosomal enzyme, (b) the human or animal lysosomal enzyme, or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions, and (II) a cleavable linker fused to the amino or carboxyl terminus of (I); and the method comprises: (a) recovering the fusion protein from the transgenic plant cell, or the cell, tissue or organ of the transgenic plant; (b) treating the fusion protein with a substance that cleaves the cleavable linker so that (I)is separated from the cleavable linker and any sequence attached thereto; and (c) recovering the separated (I). The transgenic plant can be a transgenic tobacco plant. The lysosomal enzyme can be a human or animal lysosomal enzyme. The lysosomal enzyme can be an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidese. The lysosomal enzyme can be a human glucocerebrosidase or human α-L-iduronidase. The organ can be a leaf, stem, root, flower, fruit or seed.

The present invention provides for a recombinant expression construct

plant cell. The promoter can be an inducible promoter. The inducible promoter can be induced by mechanical gene activation. The lysosomal enzyme can be a modified lysosomal enzyme which is enzymatically active and comprises: (a) an enzymatically-active fragment of a human or animal lysosomal enzyme; (b) the human or animal lysosomal enzyme or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human or animal lysosomal enzyme or (a); or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can comprise a signal peptide or detectable marker peptide at the amino or carboxyl terminal of the modified lysosomal enzyme. The detectable marker peptide 15 comprises SEQ ID NO: 10. The modified lysosomal enzyme can comprise (a) an enzymatically-active fragment of an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase; (b) the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a); or (c) the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α -L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can comprise (a) an enzymatically-active fragment of a human glucocerebrosidase or human α-L-iduronidase enzyme; (b) the human glucocerebrosidase or human α-L-iduronidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human glucocerebrosidase, human α-L-iduronidase or (a); or (c) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can be a fusion protein comprising can comprise: (I) (a) the enzymatically-active fragment of the human or

amino acid additions, deletions or substitutions, and (II) a cleavable linker fused to the amino or carboxyl terminus of (I). The lysosomal enzyme can be a human or animal lysosomal enzyme. The lysosomal enzyme can be an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase. The lysosomal enzyme can be a human glucocerebrosidase or human α-L-iduronidase.

The present invention provides for a plant transformation vector comprising any of the recombinant expression construct recited above.

The present invention provides for a plant which is transformed or transfected with any of the recombinant expression construct recited above.

The present invention provides for a plant cell, tissue or organ which is transformed or transfected with any of the recombinant expression construct recited above.

The present invention provides for a plant transfection vector comprising any of the recombinant expression construct recited above.

The present invention provides for a plasmid comprising any of the recombinant expression construct recited above.

The present invention provides for a transgenic plant or plant cell capable of producing a lysosomal enzyme which is enzymatically active, which transgenic plant or plant cell is transformed or transfected with a recombinant expression construct comprising a nucleotide sequence encoding a lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence in the transgenic plant or plant cell. The promoter is an inducible promoter. The inducible promoter is induced by mechanical gene activation. The inducible promoter comprises SEQ ID NO: 5. The lysosomal enzyme which is a modified lysosomal enzyme which is enzymatically active and which comprises: (a) an enzymatically-active fragment of a human or animal lysosomal enzyme; (b) the human or animal lysosomal enzyme or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human or animal lysosomal enzyme or (a); or (c) the human or animal lysosomal enzyme or (a) having one or more

carboxyl terminal of the modified lysosomal enzyme. The detectable marker peptide can comprise SEQ ID NO: 10. The modified lysosomal enzyme comprises: (a) an enzymatically-active fragment of an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase; (b) the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, amannosidase, sialidase or (a); or (c) the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme comprises: (a) an enzymatically-active fragment of a human glucocerebrosidase or human α-L-iduronidase enzyme; (b) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human glucocerebrosidase, human α-L-iduronidase or (a); or (c) the human glucocerebrosidase, human *a*-L-iduronidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme is a fusion protein comprising: (I) (a) the enzymatically-active fragment of the human or animal lysosomal enzyme, (b) the human or animal lysosomal enzyme, or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions, and (II) a cleavable linker fused to the amino or carboxyl terminus of (I). The transgenic plant or plant cell is a transgenic tobacco plant or tobacco cell. The lysosomal enzyme is a human or animal lysosomal enzyme. The lysosomal enzyme is an *a*-N-acetylgalactosaminidase, acid lipase, *a*-galactosidase, glucocerebrosidase, *a*-L-iduronidase, iduronate sulfatase, *a*-mannosidase or sialidase. The lysosomal enzyme is a human glucocerebrosidase or human *a*-L-iduronidase.

The present invention provides for a leaf, stem, root, flower or seed of any of the

The present invention provides for a seed of plant line Nicotiana sp., which seed has the ATCC Accession No. PTA-2155, deposited July 25, 2000.

The present invention provides for a plant grown from the seed recited above.

The present invention provides for a lysosomal enzyme which is enzymatically active and is produced according to a process comprising: recovering the lysosomal enzyme from (i) a transgenic plant cell or (ii) a cell, tissue or organ of a transgenic plant which transgenic plant cell or plant is transformed or transfected with a recombinant expression construct comprising a nucleotide sequence encoding the lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence so that the lysosomal enzyme is expressed by the transgenic plant cell or plant. The promoter can be an inducible promoter. The process is carried out with the transgenic plant and additionally can comprise a step of inducing the inducible promoter before or after the transgenic plant is harvested, which inducing step is carried out before recovering the lysosomal enzyme from the cell, tissue or organ of the transgenic plant. The modified lysosomal enzyme which can be enzymatically active and can comprise: (a) an enzymatically-active fragment of a human or animal lysosomal enzyme; (b) the human or animal lysosomal enzyme or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human or animal lysosomal enzyme or (a); or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid, additions, deletions or substitutions. The modified lysosomal enzyme can comprise a signal peptide or detectable marker peptide at the amino or carboxyl terminal of the modified lysosomal enzyme. The modified lysosomal enzyme can comprise: (a) an enzymatically-active fragment of an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase; (b) the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase, sialidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, amannosidase, sialidase or (a); or (c) the α-N-

naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme comprises: (a) an enzymatically-active fragment of a human glucocerebrosidase or human α-L-iduronidase enzyme; (b) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more amino acid residues added to the amino or carboxyl terminus of the human glucocerebrosidase, human α-L-iduronidase or (a); or (c) the human glucocerebrosidase, human α-L-iduronidase or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions. The modified lysosomal enzyme can be a fusion protein comprising: (I) (a) the enzymatically-active fragment of the human or animal lysosomal enzyme, (b) the human or animal lysosomal enzyme, or (c) the human or animal lysosomal enzyme or (a) having one or more naturally-occurring amino acid additions, deletions or substitutions, and (II) a cleavable linker fused to the amino or carboxyl terminus of (I). The transgenic plant can be a transgenic tobacco plant. The lysosomal enzyme can be a human or animal lysosomal enzyme. The lysosomal enzyme can be an α-N-acetylgalactosaminidase, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase. The lysosomal enzyme can be a human glucocerebrosidase or human α-L-iduronidase. The organ can be a leaf, stem, root, flower, fruit or seed."